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EXAMINER	
DUNSTON, JENNIFER ANN	
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1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 09/761,893	Applicant(s) HUNG ET AL.	
	Examiner Jennifer Dunston	Art Unit 1636	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *1 March 2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1,4,6,9-20,34,35,38,43 and 45* is/are pending in the application.

5a) Of the above claim(s) *12-20,43 and 45* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1,4,6,9-11,34,35 and 38* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *17 January 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☒ All b)☐ Some * c)☐ None of the:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Interim copies:

a)☐ All b)☐ Some c)☐ None of the: Interim copies of the priority documents have been received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

4) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114 was filed in this application after a decision by the Board of Patent Appeals and Interferences, but before the filing of a Notice of Appeal to the Court of Appeals for the Federal Circuit or the commencement of a civil action. Since this application is eligible for continued examination under 37 CFR 1.114 and the fee set forth in 37 CFR 1.17(e) has been timely paid, the appeal has been withdrawn pursuant to 37 CFR 1.114 and prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on 3/1/3013 has been entered.

Receipt is acknowledged of an amendment, filed 3/1/2013, in which claim 44 was canceled, and claim 1 was amended. Claims 1, 4, 6, 9-20, 34, 35, 38, 43 and 45 are pending. It is noted that the amendment filed 3/1/2013 is not compliant with 37 C.F.R. 1.121, because the period added at the end of claim 1 was not underlined, and claims 43 & 45 were not provided the proper status identifiers. Claims 43 & 45 should be identified as "Withdrawn" rather than "New." However, in the interest of compact prosecution, the amendment has been entered.

Election/Restrictions

Applicant elected Group I without traverse in the reply filed on 9/4/2001.

Claims 12-20, 43 and 45 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 9/4/2001.

Currently, claims 1, 4, 6, 9-11, 34, 35 and 38 are under consideration.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(b):

(B) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 4, 6, 9-11, 34, 35 and 38 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 1 is vague and indefinite in that the metes and bounds of the phrase “several passages” are unclear. The phrase is unclear in that the specification does not provide an explicit definition for “several.” The word "several" is defined as more than one or as consisting of an indefinite number more than two and fewer than many (Entry for "several" in Webster's Third New International Dictionary, Unabridged, Copyright © 1993 Merriam-Webster, Incorporated, Online edition, Copyright © 2001-2013 ProQuest LLC.). In the instant case, it is unclear how many passages would constitute “many” so as to define the upper bounds of the term “several.” It would be remedial to amend the claim language to clearly indicate that the “isolated mesenchymal stem cells proliferate without differentiation and reach confluence after twelve passages.”

Claims 4, 6, 9-11, 34, 35 and 38 depend from claim 1 and are rejected for the same reason applied to claim 1.

The following is a quotation of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), first paragraph:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 4, 6, 9-11, 34, 35 and 38 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor or a joint inventor, or for pre-AIA the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a new matter rejection**.

In the amendment filed 3/1/2013, claim 1 was amended to recite the additional phrase "wherein the isolated mesenchymal stem cells proliferate without differentiation and reach confluence after several passages."

The phrase "several passages" is not found in the originally filed specification. The term "several" is not defined by the specification. The word "several" is defined as more than one or as consisting of an indefinite number more than two and fewer than many (Entry for "several" in Webster's Third New International Dictionary, Unabridged, Copyright © 1993 Merriam-Webster, Incorporated, Online edition, Copyright © 2001-2013 ProQuest LLC.). The specification provides literal support for proliferation without differentiation for 12 passages (e.g., paragraph bridging pages 4-5; page 10, lines 1-3; page 14, lines 23-25; page 16, lines 1-3).

The specification also provides support for a first passage (e.g., page 13, lines 3-4; page 15, lines 4-6). Thus, the specification provides literal support for one passage, and 12 passages where the cells proliferate without differentiation. However, the claims encompass more than one or 12 passages (e.g., 13, 14...20 passages), and the specification does not provide literal or implicit support for the number of passages that would support the use of the term "several."

The reply filed 3/1/2013 acknowledges that the specification disclosed that "in one preferred embodiment of the present invention, the mesenchymal stem cells proliferate without differentiation and reach confluence even after 12 passages." However, the reply does not point to passages that provide support for "several passages."

The original specification, drawings and claims were thoroughly reviewed and no support could be found for the amendment. Accordingly, the amendment is a departure from the specification and claims as originally filed, and the passages that Applicant has provided do not provide support.

Claims 1, 4, 6, 9-11, 34, 35 and 38 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, because the specification, while being enabling for the method where small-sized red blood cells pass through pores of about 2.6 to about 6.9 μm (which specific method lacks support in the originally filed specification even though it is a species that falls within the disclosed genus), does not reasonably provide enablement for enablement of separating any small-sized cell through pores of 0.4 to 40 microns, and to be able to differentiate the mesenchymal stem cells into tissue comprising adipose or cartilage after several passages. The specification does not enable any person skilled in the art to which it pertains, or with which

it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: The claims are drawn to a method for isolating mesenchymal stem cells from bone marrow aspirate, wherein the isolated mesenchymal stem cells proliferate without differentiation and reach confluence after several passages. The method comprises the steps of (a) providing a cell mixture comprising mesenchymal stem cells and other cells; (b) seeding and culturing the cell mixture in a culture device comprising an upper plate with pores and a lower plate base, said upper plate made of a mesenchymal stem cell adhering material with pores of about 0.4 to 40 microns in diameter, where small-sized cells adhere following passing through the pores in the upper plate, said culturing with medium containing factors that stimulate mesenchymal stem cells growth without differentiation and allow for the selective adherence of only the mesenchymal stem cells to the upper plate surface; (c) purifying mesenchymal stem cells by removal of haematopoietic stem cells and non-adherent cells on the upper plate by changing medium; and (d) collecting mesenchymal stem cells from the upper plate by recovering with trypsin-EDTA, wherein the isolated mesenchymal stem cells proliferate without differentiation and reach confluence after several passages. Claim 4 requires the mixture to comprise mammalian mesenchymal stem cells, and claim 6 limits the mammalian

mesenchymal stem cells to human mesenchymal stem cells. Claim 9 requires the mesenchymal stem cells to be able to differentiate into tissue comprising bone, adipose or cartilage. Claim 10 requires the mesenchymal stem cells to be CD34-. Claim 11 limits the culture medium to 10% fetal bovine serum (FBS)-supplemented Dulbecco's'' modified Eagle's medium containing 1 g/L of glucose. Claim 34 limits the stem cell adhering material to plastic. Claim 35 requires the mesenchymal stem cells to be cultured to confluence. Claim 38 depends from claim 35 and requires re-plating the cells to expand the mesenchymal stem cells at a density of 4 x 10^3-10^4/cm^2.

The nature of the invention is complex in that the claimed range of pore sizes must allow small-sized cells to pass through while retaining mesenchymal stem cells on top. Further, the nature of the invention is complex in that after several passages, the mesenchymal stem cells must still be able to differentiate to bone, adipose or cartilage.

Breadth of the claims: The claims broadly encompass pore sizes "of about 0.4 to 40 microns in diameter" and broadly require any type of small-sized cells to pass through. The complex nature of the subject matter of this invention is greatly exacerbated by the breadth of the claims.

Guidance of the specification and existence of working examples: The specification envisions using the physical and biological characteristics of MSCs to isolate MSCs from a cell mixture. Specifically, the specification envisions using a difference in cell size and a difference in adherence to isolate MSCs (e.g., page 7, lines 10-17). The specification envisions using a plate with pores, where the pore size is defined functionally as being "sufficient for separating mesenchymal stem cells from other cells (e.g. haematopoietic stem cells)" (page 7, lines 24-27).

The specification asserts that a preferable pore size is from about 0.4 to 40 microns in diameter (e.g., page 7, lines 27-29; page 8, lines 29-30).

A working example of the claimed method is presented on page 11-12 of the specification; however, the working example **does not disclose the pore size** of the upper plate. Percoll fractionated or un-fractioned bone marrow cells in DMEM-LG with 10% FBS and antibiotics were seeded into a culture device at a density of $10^6/cm^2$ (page 11, lines 20-25). The design specifics of the culture device used in the working example are not disclosed. The specification notes that HSCs and non-adherent cells were removed with changes in medium (page 12, lines 4-6; page 14, lines 18-20). The cells retained by the upper plate were analyzed and found to have characteristics consistent with a MSC phenotype (e.g., page 12, line 16 to page 15, line 28). At page 14, the specification discusses the characteristics of the cells that are collected on the bottom plate (i.e., the cells that pass through the undefined pores). The cells were described as having a small size, polygonal shape, and little renewal capacity (page 14, lines 7-9). The specification states that the cells "were believed to be haematopoietic cells" (page 14, line 10).

The specification does not provide any characterization of the cells that are collected on the lower plate and are "believed to be haematopoietic cells." The cell surface markers of the cells were not analyzed (e.g., CD34). The ability of the cells to differentiate along the hematopoietic lineage or perform hematopoietic reconstitution is not disclosed.

Predictability and state of the art: The art teaches that pore sizes of 0.1 to 1 μm do not allow the movement of cells (US Patent Application Publication No. 2008/0085555, cited in a prior action, e.g., paragraph [0045]; US Patent No. 3,275,528, e.g., column 2, lines 23-29).

While the specification envisions the passage of haematopoietic stem cells through 0.4 μm pores, the art teaches that haematopoietic stem cells may be cultured on 0.4 μm pores, where the cells do not pass through the pores (US Patent Application Publication No. 2010/0093077, cited in a prior action, e.g., Fig. 12 and paragraph [0025]; US patent Application Publication No. 2005/0181381, cited in a prior action, e.g., paragraph [0196]). On the other hand, Schirhagl et al (Lab Chip, vol. 11, pages 3130-3135, 2011) teach that almost no large cells from bone marrow were collected with a pore size of 20 μm (e.g., Fig. 5; page 3133, right column, full paragraph). Accordingly, it would have been unpredictable to make and use the claimed method with a pore size of about 0.4 μm (the lower end of the claimed range) or with a pore size of about 40 μm (the upper end of the claimed range).

Reinhart et al (American Journal of Physiology -- Cell Physiology, vol. 248, pages C473-C479, 1985) teach that red blood cells (RBCs) pass through polycarbonate filters containing pores of 2.6 μm, 4.5 μm, and 6.9 μm (e.g., page C474, right column; page C475). Reinhart et al teach that larger white blood cells (WBCs) do not pass through the pores (e.g., page C475, left column, 2nd full paragraph).

Applicant's own post-filing art teaches that MSCs were isolated from human bone marrow aspirates by the use of a "unique method that included a specially designed culture device, which was a plastic culture dish comprising a plate with 3-μm pores to sieve out MSCs from bone marrow aspirates" (Hung et al. Stem Cells, Vol. 20, pages 249-258, 2002, cited in a prior action; e.g., page 250, right column). The specific culture device used was a 10-cm plastic culture dish comprising a plate with 3-μm pores sold as a Transwell® device by Corning Inc.

(e.g., page 251, paragraph bridging columns). The features of this device, such as the 3-μm pore size, are not taught by the present specification.

The post filing art teaches that the cells collected on the lower plate base of Hung et al have never been characterized (Zuba-Surma et al. Cytometry Part A, Vol. 75A, pages 4-13, 2009, cited in a prior action; e.g., page 11, right column, last full paragraph). Furthermore, Leong et al (Stem Cells, Vol. 22, pages 1123-1125, 2004) teaches that the teachings of Hung et al, with regard to the use of 3 μm pores to separate mesenchymal stem cells and non-mesenchymal stem cells are not convincing to the scientific communities of stem cell and tissue engineering, because the cells of the lower plate were not shown to possess different differentiation potential and characteristics as compared to the cells of the upper plate (e.g., page 1123).

It would have been unpredictable to passage the cells for 12 passages as suggested by the specification and retain the ability to differentiate the cells to a tissue that comprises adipose or cartilage. The prior art teaches that cells plated at the higher density (about 10^3 cells/cm^2) for 12 passages lose the ability to differentiate to adipocytes (Prockop et al. US Patent No. 7,374,937, cited in a prior action; e.g., column 24, lines 57-67). The present specification does not provide evidence that the cells re-plated at 4 x 10^3-10^4 cells/cm^2 for 12 passages are able to differentiate to adipose or cartilage even though the specification teaches this plating density and passage number. The specification only teaches differentiation of the MSCs 14 days following the first passage (Example 4). Other art of record teaches reduced ability to differentiate after about 19 to 21 population doublings when cells are plated at 1.5 million to 6 million cells per 150-mm dish

(Pittenger et al. Science, Vol. 284, pages 143-147, April 1999, cited in a prior action; e.g., page 145, right column).

Amount of experimentation necessary: The quantity of experimentation needed to carry out the claimed invention is large. One would be required to determine the pore sizes that provide separation of MSCs from small-sized cells obtained from bone marrow aspirate. The prior art teaches that pores of 0.1 to 1 μm do not allow cells to pass, whereas larger pores of 20 μm may allow all bone marrow cells to pass. Furthermore, the specification does not teach the pore sized used in the working example. Without the guidance of the post filing art, it would require a large amount of experimentation to determine the pore size that provides the result obtained in the specification. The post-fling art teaches a pore size of 3 μm was used, and the specification discloses a pore size of about 0.4 to 40 μm. The outcome of any experiment relying upon the guidance of the specification and prior art is unpredictable, except with regard to the removal of red blood cells with plates having pore size of 2.6 to 10 μm and the type of experimentation required is not routine in the art. Furthermore, one would be required to carry out experimentation to achieve the ability to differentiate the cells to a tissues comprising bone, adipose or cartilage at any passage number encompassed by the claimed "several" passages.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 1, 4, 6, 9-11, 34, 35 and 38 are not considered to be fully enabled by the instant specification.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 4, 6, 9, 11, 34, 35 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Reinhart et al (American Journal of Physiology -- Cell Physiology, vol. 248, pages C473-C479, 1985; see the entire reference) and Champion et al (US Patent No. 4,734,192; see the entire reference). This is a new rejection.

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by

replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x $10^3/cm^2$ (e.g., column 41). Caplan et al teach the culture of the mesenchymal stem cells without differentiation for at least one passage (e.g., column 2, lines 27-44; column 7, lines 37-67; column 8, lines 20-44; column 22, lines 34-40; paragraph bridging columns 40-41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Reinhart et al teach that red blood cells (RBCs) pass through polycarbonate filters containing pores of 2.6 μm, 4.5 μm, and 6.9 μm (e.g., page C474, right column; page C475). Reinhart et al teach that larger white blood cells (WBCs) do not pass through the pores (e.g., page C475, left column, 2nd full paragraph).

Champion et al teach a multiwall filtration apparatus comprising a microporous membrane, where the apparatus is particularly advantageous in assays requiring maintenance of fluid within the reaction wells for substantial time periods (e.g., Abstract; paragraph bridging columns 2-3; Fig. 3). Champion et al teach that microporous membrane filters and devices containing such microporous membranes have become especially useful with many of the recently developed cell and tissue culture techniques and assays (e.g., column 1, lines 24-29). Champion et al teach that the microporous membrane filter is disposed across and sealed about an aperture in a plate such that the area across each well will serve as a filtering area (e.g., paragraph bridging columns 2-3). Champion et al teach that the polycarbonate membranes are selected because of their proven characteristics in aqueous solutions and tissue culture media (e.g., paragraph bridging columns 2-3). Champion et alt each that the porosity of the membrane is selected with a view to the chosen application, with 0.025 to 10.0 micrometer porosity in a membrane of 150 micrometers thickness being preferable (e.g., paragraph bridging columns 2-3).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells in medium into the device of Champion et al for the purpose of filtering out red blood cells, because Caplan et al teach it is within the ordinary skill in the art to use a filter to remove red blood cells from cells of bone marrow aspirate, Reinhart et al teach that polycarbonate filters containing pores of 2.6 μm, 4.5 μm, or 6.9 μm allow red blood cells to pass while retaining larger, nucleated white blood cells, and Champion et al teach the use of a polycarbonate membrane with pores of 0.025 to 10 microns in diameter, where pore size is selected based upon the application. Because Caplan et al teach it is desirable to remove red blood cells, and Reinhart et al teach the appropriate pore sizes for polycarbonate membranes to separate red blood cells from nucleated white blood cells, one of skill in the art would have been motivated to include the pore sizes taught by Reinhart et al in the apparatus of Champion et al for the purpose of filtering out red blood cells. One of ordinary skill in the art would have expected a filter of about 2.6 to 10 μm to allow red blood cells to pass through while retaining the nucleated mesenchymal stem cells of Caplan et al. Furthermore, Caplan et al teach that mesenchymal stem cells are plastic-adherent. Champion et al teach that the filtration apparatus is suitable for assays where maintenance of fluid is important. Thus, one of ordinary skill in the art would have expected to successfully culture plastic-adherent mesenchymal stem cells in the medium of Caplan et al as held within the apparatus of Champion et al.

One would have been motivated to make such a modification in order to provide an enriched population of mesenchymal stem cells without the extra steps of using a column

containing a filter, or other separation method, as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the filtration apparatus of Champion et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Reinhart et al (American Journal of Physiology -- Cell Physiology, vol. 248, pages C473-C479, 1985; see the entire reference) and Champion et al (US Patent No. 4,734,192; see the entire reference) as applied to claims 1, 4, 6, 9, 11, 34, 35 and 38 above, and further in view of Pittenger et al (Science, Vol. 284, pages 143-147, 1999, cited in a prior action; see the entire reference). This is a new rejection.

The combined teachings of Caplan et al, Reinhart et al, and Champion et al are described above and applied as before.

Caplan et al, Reinhart et al, and Champion et al do not specifically teach that the mesenchymal stem cells are CD34-.

Pittenger et al teach the isolation of human mesenchymal cells from bone marrow taken from the iliac crest (e.g., page 143, right column). Pittenger et al teach that the mesenchymal stem cells are CD34- (e.g., paragraph bridging pages 143-144). The mesenchymal stem cells isolated by Pittenger et al are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to specifically use a bone marrow aspirate from human iliac crest, because Caplan et al and Pittenger et al teach the use of bone marrow from iliac crest to isolate mesenchymal stem cells that are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2). It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute iliac crest bone marrow for any other type of bone marrow to achieve the predictable result of recovering CD34- mesenchymal stem cells that are also capable of differentiating to adipose, cartilage or bone tissue.

Response to Arguments - 35 USC § 103

The prior rejections under 35 U.S.C. 103 have been withdrawn in view of the new rejections presented above.

With respect to the new rejections presented above, Applicant's arguments filed 3/1/2013 have been fully considered but they are not persuasive.

The response asserts that the specification achieved unexpected results with regard to the ability of the mesenchymal stem cells to proliferate and reach confluence even after 12 passages. The response points to Kato et al (US Patent Application Publication No. 2005/0013804), which states that "The conventional culture methods however cannot produce sufficient amounts of mesenchymal stem cells because the proliferation of said stem cells stops or becomes extremely slow around the 15^{th} generation."

These arguments are not found persuasive. In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon

which applicant relies (i.e., 12 passages) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). The claims require "several passages" but do not require "12 passages." The term "several passages" is reasonably interpreted as more than one passage given the absence of an explicit definition in the specification and the general meaning of "several." Furthermore, Prockop et al (US Patent No. 7,374,937) teaches the propagation of human bone marrow MSCs with re-plating at a density of 5 x 10^3 cells/cm^2 with growth to near confluence (e.g., column 22, lines 19-33). This is referred to as High Density Plating (e.g., paragraph bridging columns 21-22). Some MSC samples only proliferated for four population doublings, and other proliferated over 15 cell doublings from frozen cell stocks (e.g., paragraph bridging columns 23-24; Fig. 1). Differentiation assays were carried out with "late passage MSCs (e.g., passage 12)." The "late passage MSCs" retained the ability to differentiate to osteoblasts; however, the MSCs failed to differentiate into adipocytes (e.g., column 24, lines 57-67). In Example 2, Prockop et al state the following:

> The data disclosed in this example demonstrate that plating MSCs at a low density (e.g., between about 0.5 to about 10 cells per square centimeter) increases, relative to prior art culture techniques, the number of population doublings which the MSCs can undergo **without also undergoing differentiation**. As a result, differentiatable MSCs can be expanded in culture to a far greater extent than they can using prior art culture/expansion methods. (Column 27, lines 29-37). (Emphasis added.)

Prockop et al state the following at column 29, lines 27-24:

> Moreover, the **number of cell doublings is increased three-fold relative to prior art culture conditions**, as indicated in FIG. 9. Therefore, the MSC culture/expansion method described in this example, allows greater number of MSCs to be generated, and including a **greater percentage of differentiatable**

> **(i.e., multipotential) cells than do prior art culture methods**. (Emphasis added.)

Using the "low density" method, Prockop et al teach the following at column 16, lines 49-61:

> **There is no theoretical limit to the number of rounds of expansion and harvest that can be performed**. However, it is recognized that because each expansion/harvest cycle will significantly increase the number of MSCs available (i.e., by 10-fold, 100-fold, or more), a geometrically increasing amount of growth medium and growth surface will be required during sequential expansion/harvest cycles if all expanded MSCs are to be further expanded. Thus, it is recognized that for most applications, no more than about 10 cycles of expansion and harvest will normally be necessary, and as few as 1, 2, 3 or 4 cycles will be sufficient for many applications (e.g., cell therapy or gene therapy). (Emphasis added.)

Thus, the prior art successfully addressed the issue with regard to obtaining 12 passages without differentiation.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 8 am to 4 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Gussow can be reached on 571-272-6047. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Jennifer Dunston/
Primary Examiner
Art Unit 1636